EXHIBIT C
Application Serial No. 09/089,871

MAY 1993, VOLUME 14, NUMBER 5

FEATURES

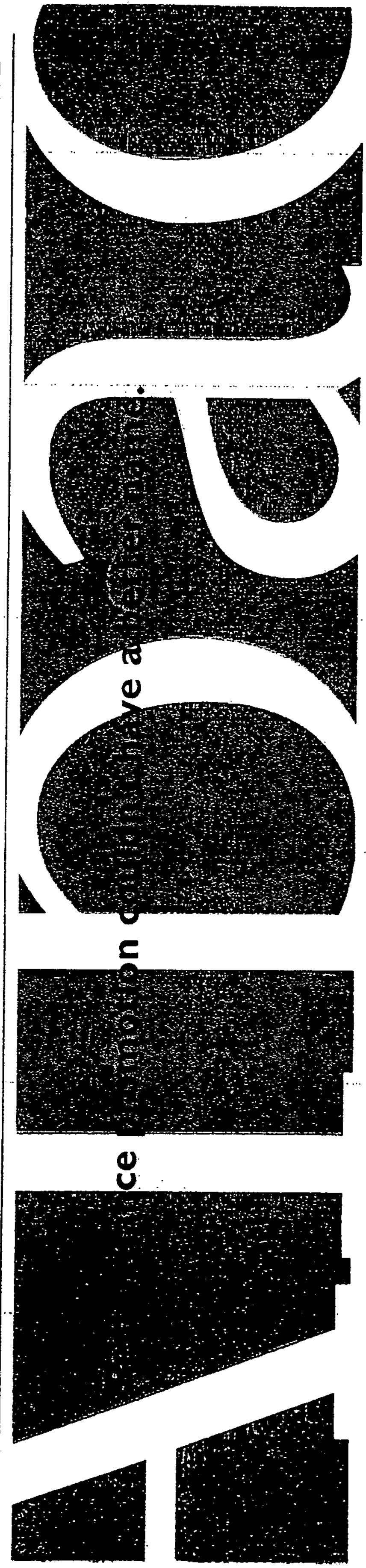
DIET DESIGN

MILL MANAGEMENT

DEPARTMENTS

On the cover: Canadian feedmill designer Luc Audet inspects the main batching scale at Coppens Mengvoeders near Helmond, Netherlands, during the Watt European Feed Tour last year.

Additional copies of this issue are available. Contact John Flowers,
Watt Publishing Co., 122 S. Wesley Ave., Mount Morris, IL 61054-1497 USA.
Tel. +1 815 734 4171 Fax +1 815 734 4201



The stability of enzymes in animal feeds

by Dr. W.D. Cowan, UK

It has been some years since feed scientists first used beta glucanase enzymes to improve poultry feeds containing barley. Since then the reliability of enzyme products has increased but has not been totally matched by a similar rise in the number of feed manufacturers and university researchers reporting good results with enzymes.

Questions arose as to the survival of enzymes in feed processing particularly, as pelleting at higher temperatures became the norm in order to destroy salmonella. Recently, feed enzyme suppliers have been expected to indicate and guarantee what amount of heat processing their products can tolerate so that the user may choose a product that can continue to function in his manufacturing system.

Focus on beta glucanases

Of the various products offered commercially, the majority are beta glucanases as this was until recently the only well established application for enzymes in monogastric feeds. In this short review, the beta glucanases are used to demonstrate how enzymes survive feed processing as by and large there is little difference in thermal stability between the various enzyme types.

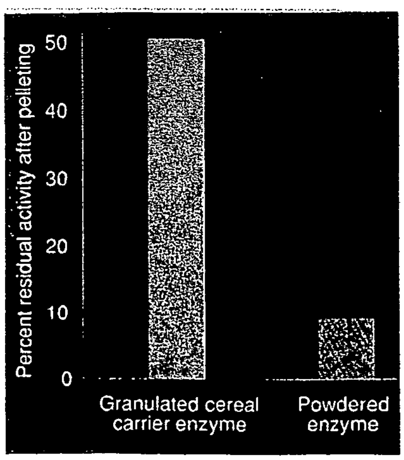


Figure 1. Stability of granulated cereal carrier-enzyme product versus powdered enzyme product during pelleting at 70°C.

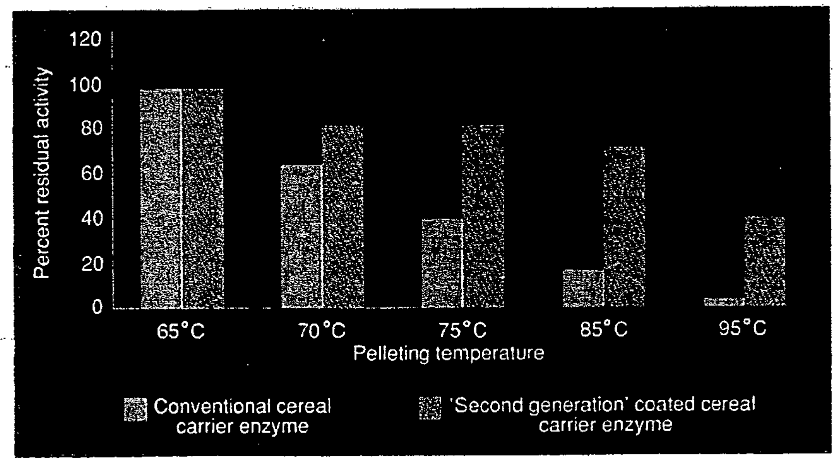


Figure 2. Stability of granulated cereal carrier-enzyme product versus 'second generation' coated granulated enzyme product at various pelleting temperatures.

Feed enzymes are normally made up of two components, the enzyme itself and a carrier material. Enzymes are proteins in which their three dimensional structure is responsible for their physical properties and catalytic activity. In all proteins, heating results in a deformation of this structure. With more heating, eventually sufficient deformation takes place and the structure is disrupted or 'denatured'. One of the most common examples of this is the coagulation of milk or egg protein in which heating renders the protein insoluble.

With enzymes, heating initially increases the catalytic activity but eventually thermal denaturation becomes more important. The active site, which is the centre of catalytic activity, is disrupted and the enzyme no longer can function. The heat used to ensure good pellet quality and to eliminate microbial activity can also denature the enzyme protein and render it inactive.

The second part of a feed enzyme is the carrier material. Pure enzymes are not used in the feed industry today because their very high activity levels would require dose levels in the range of 5-25 grams per ton of feed, making accurate dosing difficult.

The first feed enzymes were dried fermentation broths containing the enzyme, the production organism and residues from the fermentation. In addition to being non-standardised, these products were dusty and difficult to handle.

As feed enzymes developed, standardisation became necessary. The use of carriers—not only to provide support but also by diluting a stronger enzyme to a fixed level—has allowed the manufacturers to provide products of known and standard activity. Most reputable manufacturers now declare the level of enzyme activity in their products both on the label and in their product information.

Enzyme stability in processing

All enzymes are stabilised by the presence of their substrate and this has been used by enzyme manufacturers to improve the stability of their products. In Figure 1 the stability of a powder beta glucanase contrasts with that of a beta glucanase absorbed to a cereal carrier. In this case the improvement in stability is obtained by absorbing the enzyme onto its carrier, thus limiting the thermal degradation caused by steam during pelleting. Similar stabilisation techniques are used by a number of suppliers.

Absorbtion of the enzyme to a carrier improves enzyme stability by not only

---

Dr. Cowan is a nutritionist and technical advisor for Novo Nordisk, a feed enzyme supplier based at Farnham, Surrey.

bringing it into contact with its substrate but also by offering some protection against the damp heat coming from the steam in pelleting. It is this damp heat which is responsible for enzyme inactivation. Dry enzymes are much more heat resistant, being able to tolerate temperatures of 90° C for more than 30 minutes without being inactivated. The same temperature rise supplied with steam heating results in rapid inactivation.

Because of this, carrier-absorbed enzymes are not completely heat stable as can be seen from Figure 2 (p. 22).

At a pre-pelleting conditioning temperature of 65° C, a commercial enzyme absorbed to its carrier is completely stable. However, as the conditioning temperature increases, the enzyme is inactivated until at 75° C the residual activity is about 30% of the starting level. In practical application, this is about the level of activity at which it may no longer be cost effective to add enzymes to the feed. A similar pattern is seen with all of the granulated feed enzymes currently available in the market.

A second generation of feed enzymes seeks to overcome this temperature-moisture constraint. By combining existing absorption of enzyme to a carrier to a special coating process, the manufacturer has produced a new granulated enzyme product that is more resistant to pelleting. At 75° C the product has more than double the stability of other granulates as shown in Figure 3.

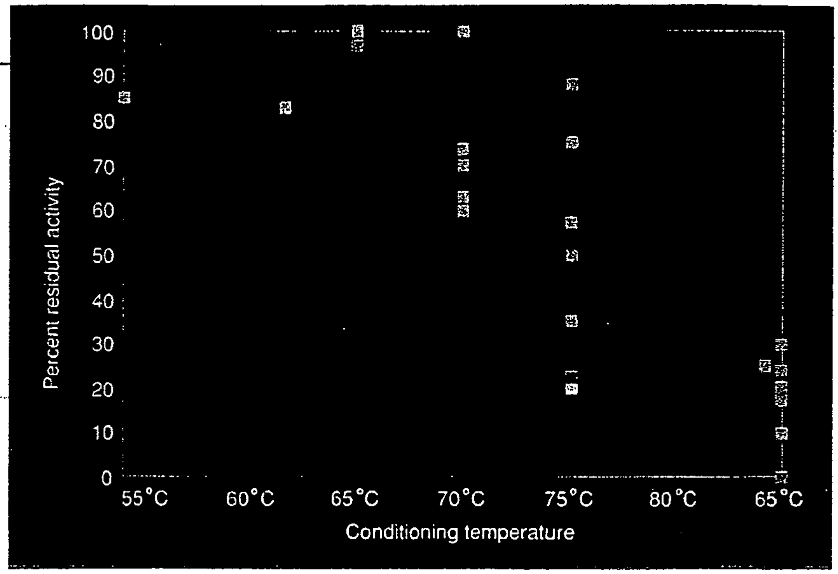


Figure 3. Stability curves of various commercial granulated enzyme products at various pre-pelleting conditioning temperatures.

Post-pelleting addition of enzymes

A feed manufacturer's concern over salmonella often leads to conditioning and pelleting temperatures above 85° C or extended conditioning times and

the use of expanders. The result of these practices is to render uneconomic even the most stable enzyme products. Liquid enzyme products are stable aqueous solutions of enzymes designed to be sprayed onto pellets after cooling. They may be applied either before or after fat coating. The small volumes required are easily absorbed even by coated feed pellets.

Slurry enzymes are suspensions of enzymes in selected vegetable oils in which the enzyme product is completely water-free. These slurry products may be blended with vegetable oil or animal fat and sprayed on in a conventional fat coating system. The absence of water means that the oil slurry enzyme is stable at higher temperatures than the conventional formulations and will survive for several hours at 80° C without significant loss of activity.

Post-manufacture chemical testing indicates that virtually 100% of the added enzyme can be recovered from the pellets with either liquid or slurry, suggesting that this can be an extremely cost effective method for enzyme addition. Practical experience at the feedmill shows that the liquid enzyme addition is easier to operate than the slurry as no melting or pre-dilution is required.

The field of enzyme applications in feed has broadened considerably since the first beta glucanase prototypes were mixed with poultry feeds. The feed manufacturer can now chose which physical form of enzyme to use depending on the pelleting conditions. Moreover, by chemically assaying for enzymes downstream from processing, the manufacturer can be assured that the feed contains sufficient enzyme to provide good animal performance. FI

Net energy: Conversion calculation

Feed ingredient evaluations based on net energy (NE) must be practicable to be used widely by feed manufacturers. Thus, Dr. Jean Noblet of the INRA national agricultural research institute in France suggests calculating the NE value of raw materials from figures for DE given in feeding tables. DE may differ significantly from metabolisable energy (ME) and net energy (NE) (see Table 1).

The first option is to use the equation NE = 0.663 x DE - 0.0039 x CP + 0.0095 x Fat = 0.0056 x CF + 0.0032 x Starch, where the result is expressed as MJ/kg dry matter. Alternatively, if the digestible nutrients content can be estimated, the equation could be NE = 0.0104 x DCP + 0.037 x DFat + 0.0148 x Starch - 0.0041 X DCF + 0.0118 X DRes, in which DRes represents digestible organic matter minus the sum of DCP + DFat + DCF + Starch.

To obtain the NE of a complete diet, Dr. Noblet says, the nutritionist can either add the contributions of ingredients or calculate directly from the crude nutrients using NE = 12.11 - 0.0282 x Ash + 0.0197 x Fat - 0.0126 x NDF + 0.0033 x Starch.

Table 1.
Comparison of energy values of some raw materials incorporated in complex pig diets.

Ingredient	DE*	ME*	NE*
Wheat	100	100	100
Barley	93	93	95
Tapioca	98	99	106
Peas	100	99	91
Soya meal	101	97	66
Animal fat	184	187	24

*As percentage of value for wheat.
Source: Adapted from Noblet *et al.* (1989, 1990)